



PATENTS

Attorney Docket No. 28200-C1

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4/8/99 Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

John J. Nestor et al.

:

App. No.: 08/453,223

: Art Unit: 1611

Filed: May 30, 1995

: Examiner: Mark L. Berch

For: 2-(2-AMINO-1,6-DIHYDRO-6-OXO-PURIN-9-YL)METHOXY-
1,3-PROPANEDIOL DERIVATIVE

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF HANS MAAG

I, Hans Maag, a citizen of the United States and Switzerland residing in Menlo Park, California, declare as follows:

I received a Diploma in Chemistry from the Federal Institute of Technology, Zurich, Switzerland, in 1969, and received a Dr.Sc.Techn. degree from the same university in October 1973. Between November 1973 and January 1975 I was a postdoctoral fellow in Chemistry at the California Institute of Technology in Pasadena, California, with Professor Robert E. Ireland. Between August 1975 and July 1985 I was employed as a chemist at Hoffmann-La Roche Inc., Nutley, New Jersey, with increasing degrees of responsibility. I have been employed as a chemist at the Syntex Research division of Syntex (U.S.A.) Inc., now

Roche Bioscience, since August 1985, and am now a Senior Research Scientist at Roche Bioscience. In the spring quarters of 1995 and 1996 and the Winter quarter of 1997, I was a Consulting Professor at the Department of Chemistry at Stanford University, and taught introductory organic chemistry to over 100 students. I have worked extensively in the synthesis of ganciclovir and its esters and related nucleosides.

I am an inventor of the invention described and claimed in this application.

I have reviewed and am familiar with US Patent No. 5,043,339 to Lilia M. Beauchamp (the "Beauchamp patent"), cited as a reference against this application.

As a part of the development of ganciclovir mono(L-valinate) as a pharmaceutical agent, I have been involved in the development of a crystalline salt of ganciclovir mono(L-valinate) that would desirably be easily preparable and stable.

I have conducted the following experiments in an attempt to prepare crystalline ganciclovir bis(L-valinate) acetate (GBVA).

Preparation of ganciclovir bis(L-valinate) acetate

[generally following Example 5 of the Beauchamp patent, on a reduced scale]

2.5 g ganciclovir, 7.54 g *N*-benzyloxycarbonyl-L-valine, and 0.4 g 4-dimethylaminopyridine were placed in a dry round-bottom flask under nitrogen, and 150 mL *N,N*-dimethylformamide (DMF) was added. 6.2 g *N,N*-dicyclohexylcarbodiimide was added to the suspension and the mixture was stirred at room temperature for ten days. The mixture was filtered and the residue was rinsed with 20 mL DMF. The filtrate was concentrated on a rotary evaporator connected to a high vacuum pump until a syrup was obtained. 100 mL dichloromethane was added and the mixture was left at room temperature overnight. 50 g silica gel (230-400 mesh) was added, and the mixture was evaporated to dryness. The dry residue was suspended in about 75 mL dichloromethane and the slurry added to the top of a column containing 300 g silica gel. The

column was eluted with 1000 mL dichloromethane followed by 500 mL each of dichloromethane containing 1%, 2%, 3%, 4%, and 5% methanol, followed by dichloromethane/6% methanol. Fractions of 150 mL (fractions 1-25) and 250 mL (fractions 26-35) were collected after the initial 1000 mL of elution. The fractions were analyzed by thin layer chromatography (10% methanol/chloroform) and fractions 19-32, containing the desired product, were combined and evaporated under reduced pressure. Drying of the residue under high vacuum overnight gave 6.41 g (89% yield) of ganciclovir bis(CBZ-L-valinate); and NMR analysis of the product showed that it contained 1 mole of DMF. To remove the DMF, the material was dissolved in 50 mL dichloromethane and washed five times with 100 mL water, with the washings being separately back-extracted with 50 mL dichloromethane. The organic extracts were combined, dried over magnesium sulfate, and concentrated on a rotary evaporator. The residue was dried at room temperature under high vacuum for two hours to provide 5.96 g (82.5% yield) of ganciclovir bis(CBZ-L-valinate) as a white solid. NMR analysis showed the presence of the desired product with only very minor amounts of DMF and dichloromethane.

5.94 g ganciclovir bis(CBZ-L-valinate) was dissolved in 70 mL glacial acetic acid and placed in a Parr flask. 2.1 g 10% Pd on carbon was added and the mixture was hydrogenated for 16 hours under an initial hydrogen pressure of 50 psig. The resulting mixture was filtered through Celite and the residue rinsed with glacial acetic acid. The filtrate was evaporated on a rotary evaporator connected to a high vacuum pump. The oily residue was dried under high vacuum for five hours. The residue was dissolved in 100 mL water and lyophilized over the weekend. After drying, the residue was scraped out of the flask, yielding 4.4 g material, which was transferred to a vial and dried at 100°C over phosphorus pentoxide for 19 hours, resulting in a glass (3.64 g). NMR analysis of this material, showing a number of additional signals inconsistent with the desired product (e.g. multiple signals in the range of 7.2-8.2 ppm, where a singlet is expected for H-8 of the guanine ring, and low relative integration of the acetic acid methyl groups) indicated partial decomposition and/or partial removal of acetic acid during drying. Elemental analysis showed: C 48.51; H 6.58; N 18.35; calculated for ganciclovir bis(L-valinate). $2\text{CH}_3\text{COOH}$: C 48.16; H 6.85; N 17.05.

Further preparation of ganciclovir bis(L-valinate) acetate

5.1 g ganciclovir, 15.1 g *N*-benzyloxycarbonyl-L-valine, and 0.8 g 4-dimethylaminopyridine were placed in a dry round-bottom flask under nitrogen, and 300 mL *N,N*-dimethylformamide (DMF) was added. 12.4 g *N,N*-dicyclohexylcarbodiimide was added to the suspension and the mixture was stirred at room temperature for two days. The mixture was filtered and the residue was rinsed with 50 mL DMF. The filtrate was concentrated on a rotary evaporator connected to a high vacuum pump until a syrup was obtained. The oily residue was suspended in 200 mL dichloromethane, and washed twice with 100 mL water, resulting in a thick emulsion of which the two layers separated very slowly and incompletely. The major part of the dichloromethane layer was drained into an Erlenmeyer flask and dried over magnesium sulfate. The dichloromethane was allowed to evaporate. 200 mL fresh dichloromethane was added and the resulting mixture was filtered. The residue was rinsed with dichloromethane, and the filtrate and rinsings concentrated on a rotary evaporator to dryness. The residue was dried under high vacuum at room temperature for 20 hours to give 19.6 g ganciclovir bis(CBZ-L-valinate) as a foam.

8.45 g ganciclovir bis(CBZ-L-valinate) was dissolved in 100 mL glacial acetic acid and placed in a Parr flask. 3.0 g 10% Pd on carbon was added and the mixture was hydrogenated for 16 hours under an initial hydrogen pressure of 50 psig. The resulting mixture was filtered through Celite and the residue rinsed with 30 mL glacial acetic acid. The filtrate was evaporated on a rotary evaporator connected to a high vacuum pump, and the oily residue was dried under high vacuum for four hours. The residue was dissolved in 300 mL water and lyophilized for two days, resulting in an amorphous white solid. The residue was dissolved in 75 mL water and lyophilized for three and one-half days, resulting in an oily residue. The solid amorphous residue was broken up and again dried under high vacuum for four hours, yielding 6.2 g (91.5% yield) of ganciclovir bis(L-valinate) acetate as an amorphous solid. NMR analysis indicated a high purity for the material (single H-8 peak at 7.81 ppm), and the presence of 2.6 equivalents of acetic acid, which was substantiated in the elemental analysis: found: C 47.18 and 47.18; H 6.96 and 7.12;

N 15.76 and 16.00; calculated for ganciclovir bis(L-valinate).
2.6 CH₃COOH. 0.34 H₂O (MW 617.65): C 47.18; H 6.89; N 15.88.

**Preparation of ganciclovir bis(L-valinate) acetate
[generally following Example 5(b) of the Beauchamp patent at a larger
scale]**

3.084 g ganciclovir bis(CBZ-L-valinate) was dissolved in 35 mL glacial acetic acid under nitrogen. 0.9 g 10% Pd(OH)₂ on carbon was added, and the mixture was hydrogenated (initial pressure 40 psi) on a Parr shaker for fifteen hours. The mixture was filtered through Celite, and 15 mL glacial acetic acid was used for rinsing. The filtrate was evaporated on a rotary evaporator connected to a high vacuum pump (bath temp. 35°C) to an oily film. The oily residue was dried under high vacuum for two hours, leaving 4.3 g of an oil containing GBVA.

The oil was dissolved in 50 mL deionized water and the slightly milky solution was lyophilized overnight, resulting in 2.5 g of a fluffy white solid. The solid was dissolved in 40 mL deionized water; and the slightly milky solution was filtered, and the filtrate again lyophilized overnight, giving 2.2 g GBVA as an amorphous solid. The non-crystalline nature of this material was confirmed by an X-ray powder diffraction analysis, which showed no distinctive bands. Elemental analysis of the material showed: C 47.34 and 47.22; H 6.83 and 6.80; N 16.48 and 16.40; calculated for ganciclovir bis(L-valinate). 2 CH₃COOH. 0.6 H₂O: C 47.27; H 6.93; N 16.78. NMR analysis indicated no significant impurities and the presence of 2 equivalents of acetic acid. LC/MS analysis indicated a purity of 98.27% by evaporative scattering light detection, with the major peak showing the correct mass (454 = M+H-2HOAc).

This sample of GBVA was subjected to the following crystallization attempts (dissolution followed by addition of less polar solvents accompanied with scratching with a spatula to induce crystallization):

(1) 154 mg GBVA was dissolved in 0.5 mL isopropanol and 0.5 mL water. To the clear solution was slowly added 2.0 mL isopropanol, followed by 4.0 mL acetone, followed by 2.0 mL ether. The ether addition was accompanied by temporary cloudiness. The mixture was left at room temperature for one hour, then placed in a refrigerator overnight. The GBVA formed an oil.

(2) 168 mg GBVA was dissolved in 0.75 mL methanol, and 2.0 mL ethyl acetate was slowly added. After one hour at room temperature, an additional 1.0 mL ethyl acetate was added. The solution suddenly became cloudy, and an amorphous precipitate of GBVA formed.

(3) 1.5 mL isopropanol was added to 158 mg GBVA, which only partially dissolved. 1.0 mL methanol was added to aid solubilization, yet not all the GBVA dissolved. The mixture was stirred for four hours at room temperature, with no change, and an additional 2.0 mL methanol was added, resulting in a clear solution. The mixture was left overnight before further addition of isopropanol was attempted. The mixture was left at room temperature for four hours, at which time a few needles appeared. The flask was placed in a refrigerator overnight, which resulted in the deposition of an oily amorphous material over the needles. The mixture was briefly warmed to attempt crystallization, but this was unsuccessful. The supernatant was removed by pipette and the residue was rinsed twice with ether. Drying resulted in 3 mg of a mixture of crystalline and amorphous material. The melting point of this material was measured on a hot-stage polarizing microscope. The amorphous material shrank continuously on heating, with true melting starting at approximately 178°C. The material melted over a wide range, with the amorphous melting first (to approximately 185°C) and the last crystalline specks melting at 188°C. NMR analysis of this material showed it to be a mixture of compounds (at least two H-8 signals) and no acetic acid methyl group was clearly discernible. However, LC/MS showed this material to be not significantly different from the hydrogenation product. From these data I conclude that the crystalline material is a neutral or partial

salt form of ganciclovir bis(L-valinate) and not GBVA, which explains the formation of only a small amount of crystalline material and the lack of progressive crystallization. I would not expect this material to be stable, based on the pH stability profile of ganciclovir valine esters.

(4) 132 mg GBVA was dissolved in 1 mL methanol to form a clear solution. 3 mL tetrahydrofuran followed by 2 mL ether were added slowly. The solution suddenly became cloudy and an amorphous precipitate formed.

(5) 146 mg GBVA was dissolved in 0.7 mL methanol and 1.5 mL chloroform was added. After four hours at room temperature, 2 mL ether was added slowly, resulting in a temporary cloudiness during addition. The mixture was left at room temperature, and a clear oil separated from the solution.

(6) 138 mg GBVA was dissolved in 0.7 mL methanol. 1.5 mL toluene was added slowly. After four hours at room temperature, 1.5 mL hexane was added slowly. An oil formed, which turned into an amorphous solid on standing.

Conclusion

In my opinion, based on my knowledge of crystallization of organic compounds, in particular acid addition salts, and especially ganciclovir esters, the inability of myself and others to prepare crystalline ganciclovir bis(L-valinate) acetate, and the inability of others to prepare crystalline ganciclovir mono(L-valinate) acetate, I believe that it is not possible to prepare ganciclovir bis(L-valinate) acetate in stable crystalline form because acetic acid is a weak acid with a low dissociation constant ($K_a \sim 1.8 \times 10^{-5}$), so that it does not form strongly bound acid addition salts; and I consider that this will lead to a dynamic mixture of ganciclovir bis(L-valinate) acetate salt forms in different protonation states, which are detrimental to crystallization.

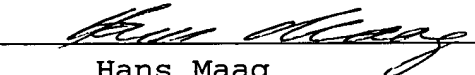
For this reason, I believe that ganciclovir bis(L-valinate) acetate will not form a stable crystalline compound; but rather will

form a compound of variable composition depending on the conditions of preparation, and this compound will be unstable to, for example, gain or loss of solvent of crystallization, so that it may be hygroscopic and/or deliquescent.

Ganciclovir bis(L-valinate) acetate as prepared is non-crystalline, and repeated attempts to prepare it in crystalline form using conditions calculated to cause crystallization of the material have been unsuccessful. I believe that it is not possible to prepare ganciclovir bis(L-valinate) acetate in crystalline form.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under 18 USC 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 6, 1999


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